

OTRI. KASUMI-1 CELL LINE: AN ALTERNATIVE TO THE POTENCY ASSAY OF FILGRASTIM BIOPHARMACEUTICAL.

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INTRODUCTION Filgrastim is classified as a biopharmaceutical and it is the cytokine G-CSF (Granulocyte-Colony Stimulating Factor) human recombinant protein. The biological effect of this cytokine G-CSF is mainly related to the proliferation and differentiation of granulocytes, which are important cells in host defense against many microorganisms. Neutropenia caused by chemotherapy or as a consequence of diseases are widely treated with this recombinant protein. Filgrastim is part of the list of drugs that can be purchased by the Brazilian health system (SUS) and this medicine will soon be produced at Fiocruz. The relationship between potency and efficacy of recombinant proteins is very strong and any variations in this respect may compromise the wished effect in patients. Moreover, physicochemical assays alone does not assure the pharmacological effect of this type of substance. Therefore, quality control procedures must be designed to confirm the biological effect and be performed routinely. Currently, there is no method in the national pharmacopoeia for this biopharmaceutical. In the European Pharmacopoeia, the pharmacological potency is recommended to be determined by an *in vitro* assay performed in M-NFS-60 cell line and is based on the evaluation of proliferative potential, as measured by MTT metabolization. This cell line, however, has some disadvantages such as: the need for supplementation with M-CSF and it is a mouse cell line.

OBJECTIVE In this context, we aim to develop *in vitro* potency assay with a human cell line, Kasumi-1, using the MTT cell proliferation test. Specific objectives: Standardize the culture of Kasumi-1 cells; use different cell concentrations to test the medicine; evaluate different concentrations of Filgrastim in the MTT assay;

METHODOLOGY Cell culture - Kasumi-1 was maintained in RPMI-1640 supplemented with 20% FCS maintained at 37°C with 5% CO₂. MTT proliferation assay - based on the criteria established in European Pharmacopoeia, viable cultured

cells are plated with medium in 96-well microplates. Subsequently, cells are exposed to different concentrations of the drug. After the incubation, MTT was added and absorbation was measured using a spectrophotometer 570nm .

RESULTS Two initial concentrations were used. 800 IU/ml as European Pharmacopoeia, with serial dilution 1: 2, and another curve starting in 100000 IU/ml, with serial dilution 1: 5. Both showed a tendency of a dose-dependent curve, indicating a potency relationship. The curve analysis was performed using a potency curve and the coefficient of determination (r) was higher than 80%.

CONCLUSION The preliminary data obtained with Kasumi-1 cell line suggests that this cell might be useful in the determination of potency of filgrastim. More studies, however, must be performed to assure the feasibility of the proposed assay.

KEYWORDS potency assay, quality control, biopharmaceutical.